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TITLE: Functional Disruption of the Netrin-1 Guidance Cue Leads to Disruption in Mammary Gland Development and Increased Tumor Incidence

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<b>13. ABSTRACT (Maximum 200 Words)</b> My laboratory discovered a mammary receptor for netrin-1 called neogenin, and I proposed to examine the stromal phenotype associated with the loss of function of neogenin using transplant analysis. Analysis of homozygous null mammary tissue versus wildtype revealed no changes in tissue morphology due to the loss of neogenin function during adolescent development. The investigation is still underway as additional stages of mammary gland development are analyzed. These experiments will extend our study of netrin-1 and its receptor neogenin during mammary gland development, and potentially yield insights into the role netrin-1 plays in mediating stromal/epithelial interactions during normal breast growth and malignant breast disease.				
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**Introduction:**

In the first year, we discovered a mammary receptor for netrin-1. On the basis of this discovery, the advice of my grant reviewers and the recommendation after my first annual summary, I revised my Statement of Work (SOW) to focus my studies on the function of this receptor during mammary gland development. I specifically proposed to examine the stromal phenotype associated with the loss of neogenin using transplant analysis. To facilitate these experiments, I first backcrossed neogenin  $-/+$  mice 12 generations to generate a syngeneic background. Next I examined the stromal phenotype combining neogenin  $-/-$  stroma with both  $+/+$  and  $-/-$  epithelium during the adolescent stage of development and observed no apparent phenotype compared to wildtype. I am continuing these studies by examining the phenotypes in mature animals during pregnancy, lactation and involution.

**Body:** I revised and received approval for Task 3 of my SOW to be completed during months 12-24. I proposed to analyze neogenin null mammary glands at all developmental stages: adolescent, pregnancy, lactation and involution using transplant analysis. To facilitate this study, I created syngeneic neogenin  $+/-$  mice in which to perform the transplant analysis. I transplanted the tissue as outlined in my SOW, combining neogenin  $-/-$  stroma with both  $+/+$  and  $-/-$  epithelium and compared this to contralaterally transplanted  $+/+$  stroma;  $+/+$  epithelium control. During the adolescent stage of development, I harvested the tissue and examined it histochemically. No differences were observed between neogenin  $-/-$  and  $+/+$  tissue, suggesting that neogenin does not play a significant role in mediating outgrowth of the gland. The analysis of additional stages (pregnancy, lactation and involution) is currently underway.

**Key Research Accomplishments:**

- Generated syngeneic neogenin  $+/-$  mouse strain.
- Transplanted  $+/+$  and neogenin  $-/-$  epithelium into neogenin  $-/-$  stroma and compared this to wildtype control.
- Analyzed the transplants during adolescent development and observed no changes in development due to loss of neogenin function.

**Reportable Outcomes:***Presentations:*

Lindsay Hinck, Grace Shin, Phyllis Strickland, 2004. *The role of axon guidance molecules in mammary gland development*, Basement Membrane Gordon Conference, New Hampshire

Lindsay Hinck, Grace Shin, Phyllis Strickland, 2004. *The role of axon guidance molecules in mammary gland development*, Mammary Gland Gordon Conference, Italy

Lindsay Hinck, Grace Shin, Phyllis Strickland, 2004 *The role of axon guidance molecules in mammary gland development*, Keystone Symposium, Signaling in Vertebrate Organogenesis, New Mexico

*Funding Received:*

California Breast Cancer Research Program, 2004-2007: total award \$449,228

In performing these experiments this year, I started to think about the role other axon guidance proteins play during normal breast development and disease. Another family of proteins that function during neural development as guidance molecules are the Slits and their Robo receptors. Loss or misexpression of these genes has been implicated in tumor progression in a variety of target organs, including breast. To pursue these studies, I wrote and received a new grant from the California Breast Cancer Research Program.

**Conclusions:**

The investigation into the role of neogenin in stromal tissue during mammary gland development in the adolescent animal revealed no changes in tissue morphology due to loss of neogenin function. The investigation is still underway as additional stages of mammary gland development are being analyzed. These experiments will extend our study of netrin-1 and its receptor neogenin during mammary gland development, and potentially yield insights into the role netrin-1 plays in mediating stromal/epithelial interactions during normal growth and malignant disease.